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Short-term effects of low protein-normal sodium diet on renal function in chronic renal failure

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Short-term effects of low protein-normal sodium diet on renal function in chronic renal failure. To investigate the short-term renal effects of protein restriction and unchanged salt intake in chronic renal failure (CRF), patients with moderate CRF (creatinine clearance 41 ± 5 ml/min) and healthy controls (CON) ate a normal protein diet (NPD) for four weeks, and thereafter a low protein diet (LPD, 0.4 g/kg body wt/day) for three weeks. The two diets were isocaloric and with a salt intake of 10 to 13 g/day. No differences in body weight, serum proteins and plasma sodium were recorded throughout the study. During LPD, inulin and PAH clearances in CON demonstrated a progressive 25% decline of basal GFR and RPF; on the contrary, in CRF, basal renal function did not change in presence of a significant reduction of proteinuria. In CON patients after protein restriction, fractional free-water generation (C_{H_2O}/C_{In}) and fractional urinary excretion of sodium (FE_{Na}) measured under maximal water diuresis increased progressively, both being doubled at the end of LPD, while in CRF, C_{H_2O}/C_{In} did not change and FEN_a values remained unmodified and much higher (above 4%) than in CON after both diets. The renal response to an acute oral protein load (OPL) and i.v. low-doses of dopamine (D) was measured at the end of each period; in the two groups, GFR and RPF significantly increased following OPL + D after both diets. In CRF, however, the vasodilatory response was blunted overall being reduced after both LPD and NPD, and, unlike CON, it did not increase after LPD. In conclusion, the data indicate that in moderate CRF: (a) in the first three weeks of protein restriction and constant salt intake (10 to 13 g/day), basal renal dynamics do not change; (b) the renal response to an acute vasodilatory stimulus is reduced and not influenced by dietary protein levels; (c) the abnormal response of basal and stimulated renal function to LPD may be related, at least partially, to a very high distal delivery of sodium preventing the normal effects of proteins on the tubuloglomerular feedback system.

In chronic renal failure (CRF), a reduced protein intake has been proposed to improve glomerular permselectivity, diminish the level of proteinuria and delay end-stage renal disease [1–3]. The mechanisms underlying the negative effect of proteins are still ill-defined; however, micropuncture studies in rats have suggested that high protein intakes lead to a condition of glomerular hyperfiltration, that is, increased glomerular pressure and flows, which is hypothesized to be responsible for development of glomerular sclerosis and decreasing renal function [4]. Therefore, different investigators have evaluated short-term effects of low-protein diets (LPD) in normal subjects [5, 6] as well as in CRF patients [1, 7–12]; however, these studies appear not to be conclusive. Indeed, although a decreased proteinuria following LPD is shown, the data on renal dynamics are contradictory, indicating either absence [1, 5] or presence of a positive correlation between dietary proteins and renal perfusion [6–12]. Furthermore, it is important to note that in most of the previous papers the compliance to the assigned protein intake was not carefully verified by repeated urinary measurements. The daily salt intake was also not ascertained nor maintained constant during LPD despite the demonstration, by recent studies in experimental animals, that dietary sodium contributes not only to regulation of basal GFR but to the pathogenesis of glomerulosclerosis in CRF [13].

In 1983, to detect presence of glomerular hyperfiltration in humans, Bosch et al proposed to measure the renal functional reserve (RFR), that is, the capacity of the kidney to increase renal plasma flow and GFR in response to an acute vasodilatory stimulus [7, 14]. According to these authors, a reduced RFR would witness the presence of glomerular hypertension. Their original hypothesis has not been confirmed by recent micropuncture studies demonstrating that a reduced RFR is linked to an abnormal proximal tubular reabsorption rather than to glomerular hyperfiltration [15–18]. Clinical studies have tested renal reserve in chronic renal failure with conflicting results demonstrating either diminished [7, 10, 12, 19] or normal response [11, 20–22]. The observed discrepancies are likely related to the lack of a standard protocol, as well as to the presence of methodological pitfalls that make the interpretation of the data difficult. Some of these studies in fact lack an appropriate control group [11, 12]. Moreover, in different reports GFR has been estimated by creatinine clearance [10, 14, 20], while it is now well established that an acute protein load directly alters tubular secretion of creatinine [22–24]. Finally, in most of the previous studies, sodium and water intake was not ascertained, although the volume status and the level of hydration greatly influence the renal response to acute protein loads [24, 25].

In the present study, we consecutively administered to normal subjects and patients with established proteinuric renal failure two diets differing in protein intake only. The aim was to

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investigate, under maximal water diuresis conditions, the short-term effects of protein restriction on basal renal dynamics, tubular reabsorption in the proximal nephron and renal functional reserve in presence of unchanged salt intake.

Methods

Patients

The study was performed in 14 patients with chronic renal failure and 6 healthy volunteers. Patients (7 males, 7 females) averaged 44 years old (range 22 to 65); normal subjects (3 males, 3 females) averaged 38 years of age (range 30 to 50). We excluded from the study patients with diabetes mellitus, neoplastic diseases, heart failure, liver failure, nephrotic syndrome, hypertension with a diastolic blood pressure constantly higher than 105 mm Hg, salt losing nephritis, urinary tract obstruction or other reversible causes of renal failure. Kidney biopsy was performed in seven patients, showing interstitial nephritis in four of them and membranoproliferative glomerulonephritis in three patients; radiologic techniques evidenced policystic kidney disease in one patient, while the other six patients clinically had primary glomerular disease. During the study, patients were not taking any medication on a regular basis. Healthy volunteers had no history of renal disease, hypertension or diabetes. At the time of the study, the urine dipstick was negative for blood, protein, glucose, and blood pressure was normal. The initial GFR, evaluated by creatinine clearance, in healthy subjects and in patients averaged 124 ± 8 ml/min and 41 ± 5 ml/min, respectively.

Study protocol

Prior to the study, urinary measurements and interviews with a dietician allowed an assessment of the diet normally followed by CRF patients and controls. The results of these preliminary studies evidenced a protein intake of 1.5 g/kg body wt/day in CRF and 2 g/kg body wt/day in controls with similar salt (10 to 13 g NaCl/day) and calorie (40 to 45 Kcal/kg body wt/day) intake. The study lasted seven weeks. During the first four weeks, both groups of subjects were followed as out-patients; they were asked to follow their usual diet and to describe the daily meals in a diary. On weekly basis, they were interviewed by a dietician to control compliance to the assigned diet, and moreover, 24-hour urinary samples were collected weekly to measure salt and protein intake. On the last day of this period (normal protein diet, NPD), patients and control subjects were hospitalized and switched to a lower protein intake (protein prescribed: 0.4 g/kg body wt/day) lasting three weeks (LPD). During LPD, the dietary content of proteins and sodium was ascertained daily by a 24-hour urinary collection. The two diets contained the same amount of NaCl and were isocaloric. In patients, the calorie intake at NPD and LPD was 39 ± 2 and 40 ± 2 Kcal/kg body wt/day, respectively, and, in normal subjects, 45 ± 1 and 44 ± 1 Kcal/kg body wt/day, respectively. During the last three weeks of the study, inulin and PAH clearances were measured at days 0, 3, 6, 9, 12, 15, 21 in controls and ten patients. RFR was assessed at the end of each period of diet in all the subjects.

Renal function and renal reserve determination

Renal function determination was performed at the same time of day during a steady state of maximal water diuresis, obtained by oral water loading, to gain insights into the tubular function of the proximal nephron. On the morning of the test, fasting subjects drank 15 ml/kg body wt of tap water. Thereafter, an amount of water equal to the urinary volume collected minus the amount administered with the infusion solution was given orally to maintain water balance. To perform i.v. infusions and blood sampling, small Teflon cannulae (Abbot Labs., Illinois, USA) were inserted into an antecubital vein of each arm. A bolus injection of a priming dose of inulin (50 mg/kg body wt; Jacopo Monico, Venezia/Mestre, Italy) and sodium paraminohippurate (PAH, 10 mg/kg body wt; Jacopo Monico) in a 50 ml of saline solution was performed. Thereafter, a continuous infusion (1 ml/min) of inulin (125 mg/creatinine clearance/500 ml 5% D-solution) and PAH (12.5 mg/estimated RPF/500 ml 5% D-solution) was started and continued throughout the experiment in order to maintain constant the plasma concentration of the two markers. After 60 minutes of stabilization, three clearance periods of 30 minutes each were obtained. Blood was withdrawn at the beginning and at the end of each period through a catheter kept open by a flushing of heparinized solution. In all the subjects studied, in which a post-voiding urinary volume was previously excluded by ultrasound, urine collection was obtained by spontaneous voiding. Blood pressure was measured during each clearance period.

On the last day of each diet, patients and control subjects, after the stabilization period, underwent a one-hour clearance period to measure basal GFR and RPF. Then they were given the vasodilating stimulus to quantify the renal functional reserve. It has been previously reported that maximal stimulation of GFR is obtained by amino acid load plus dopamine infusion [19]. Therefore, we acutely administered an oral protein load as a lean cooked beef steak (4.4 g/kg body wt; range: 3.5 to 6.5 g/kg, corresponding to 0.88 g protein/kg body wt) ingested in 30 minutes associated to continuous infusion of vasodilating doses of dopamine (1 μg/kg/min). After the protein load and during dopamine infusion, three clearance periods of one hour each were obtained for GFR and RPF measurements.

Calculations

GFR and RPF were corrected for body surface area. RPF was calculated by dividing the corresponding PAH clearance by an estimate of the renal extraction ratio of PAH. According to other authors that have examined subjects with GFR values similar to those recorded in our study, the renal PAH extraction ratio was assumed to be 0.85 in healthy subjects, and 0.70 in patients with chronic renal failure [22].

Protein intake was calculated from the following equation [26]: Estimated protein intake (g/day) = 6.25 * [UUN (g/day) + SBW (kg) * 0.031 (g/kg SBW/day)]. This formula was used also in subjects with normal renal function even though this may slightly underestimate the protein intake since, in normals, greater amount of urinary nitrogen may be lost as ammonium.

Laboratory procedures

Plasma and urinary concentrations of proteins, nitrogen, sodium, potassium, osmoles, inulin and PAH were analyzed using standard techniques described in previous papers [27, 28].

Table 1. Clinical data of the last day of normal protein diet (NPD) and low protein diet (LPD)

	Patients (N = 14)		Controls (N = 6)	
	NPD	LPD	NPD	LPD
Body weight *kg*	62.7 ± 2.0	62.4 ± 2.0	68.8 ± 1.7	67.9 ± 1.8
Hematocrit %	38.7 ± 1.5[a]	37.7 ± 1.3[a]	45.3 ± 1.0	43.5 ± 0.9
Serum urea nitrogen *mg/dl*	38 ± 5[a]	9 ± 1[ab]	12 ± 2	2 ± 1[b]
Serum creatinine *mg/dl*	2.2 ± 0.3[a]	2.2 ± 0.3[a]	0.8 ± 0.1	0.8 ± 0.1
Serum bicarbonate *mEq/liter*	19.5 ± 0.9[a]	21.1 ± 0.3[a]	26.1 ± 0.3	28.7 ± 0.9
Total protein *g/liter*	7.2 ± 0.2	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1
Serum albumin *g/liter*	4.4 ± 0.1	4.2 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
Serum osmolality *mOsm/liter*	300 ± 3[a]	291 ± 2[ab]	286 ± 4	284 ± 4
Plasma sodium *mEq/liter*	140 ± 1	140 ± 1	141 ± 2	142 ± 1

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ vs. NPD

Table 2. Tubular function at the last day of normal protein diet (NPD) and low protein diet (LPD)

	Patients (N = 14)		Controls (N = 6)	
	NPD	LPD	NPD	LPD
V *ml/min*	6.0 ± 0.6[a]	5.3 ± 0.4[a]	9.8 ± 1.4	10.1 ± 2.7
FE_{Na} %	4.6 ± 0.6[a]	4.5 ± 0.6[a]	1.2 ± 0.2	2.4 ± 0.1[b]
FE_K %	31 ± 4	27 ± 3	18 ± 3	21 ± 2
U_{Osm} *mOsm/kg* H_2O	157 ± 24	105 ± 12[b]	123 ± 10	85 ± 2[b]
C_{H_2O}/C_{In} %	8.6 ± 1.8	9.5 ± 0.9	4.1 ± 0.1	9.2 ± 0.9[b]

Abbreviations are: V, urinary volume; FE_{Na}, fractional urinary sodium excretion; FE_K, fractional urinary potassium excretion; U_{Osm}, urinary osmolality; C_{H_2O}/C_{In}, fractional free-water generation.
[a] $P < 0.05$ vs. Control
[b] $P < 0.05$ vs. NPD

Statistics

All values are reported as mean ± SEM. We used one-way analysis of variance for comparisons among different conditions, and ANOVA for repeated measurements to analyze differences in the same group. Regression analysis was also performed. The level of statistical significance was defined as $P < 0.05$.

Results

Tables 1 and 2 report basal data recorded in patients (CRF) and controls (CON) on the last day of each dietary period. In both groups, no difference was detected in body weight (body wt), serum total protein (TP), serum albumin (S_{Alb}), serum sodium (S_{Na}), serum osmolality and basal urinary output (V) between the two dietary periods. These data therefore demonstrate that nutritional status as well as volume and hydration state did not vary. Basal hematocrit (Htc) was significantly lower in CRF. All the renal dynamic studies were performed in a state of maximal water diuresis, which was demonstrated by the achievement of minimum values of urinary osmolality (Table 2).

Compliance with the prescribed diet was demonstrated by the constancy of urinary sodium excretion and the significant decrease of urinary urea nitrogen excretion observed after reduction of protein intake. In each single subject of both groups, the variance of urinary measurements was not greater than 20%. In CRF patients (Fig. 1), sodium excretion was constant, averaging 188 ± 17 mEq/day in the first period (NPD, normal protein diet) and 179 ± 15 mEq/day during low protein diet, LPD (NS). In NPD the mean urinary urea nitrogen was 9.8 ± 0.6 g/day, corresponding to an average protein intake of 1.43 ± 0.1 g/kg body wt/day while in LPD the same value decreased to 3.0 ± 0.2, corresponding to 0.46 ± 0.02 g/kg body wt/day of proteins. Similarly, as shown in Figure 2, controls had a sodium excretion of 229 ± 45 mEq/day in NPD and 210 ± 34 in LPD, while the urinary urea nitrogen decreased from 11.9 ± 1 g/day to 3.9 ± 0.1 g/day, corresponding to a protein intake which went from 1.91 ± 0.1 g/kg body wt/day to 0.41 ± 0.02 g/kg body wt/day. No significant difference was detected between the two groups in sodium excretion and the percent decrease of urinary urea nitrogen.

Figure 3 shows the progressive changes in GFR, RPF and FE_{Na}, measured during maximal water diuresis, following the decrease in protein intake in the two groups of subjects studied. When protein content of the diet was reduced in normal subjects, GFR progressively decreased, being 139 ± 7 ml/min at the end of NPD period and 105.6 ± 8 ml/min at the end of LPD period (−24%, $P < 0.01$). The reduction in GFR was related to a similar decrease in renal perfusion; indeed, RPF was 697 ± 46 ml/min at NPD and 515 ± 29 ml/min at LPD (−26%, $P < 0.01$). The reduction of GFR and RPF was already significant at the sixth day of the LPD diet. As opposed to healthy subjects, patients with chronic renal failure did not present any significant variation in basal renal dynamics following the change of dietary proteins. Interestingly, in CON at normal protein diet, the higher basal values of GFR and RPF were associated with lower values of sodium fractional urinary excretion (FE_{Na}) as well as of fractional free-water generation (C_{H_2O}/C_{In}). As shown in Figure 3c, FE_{Na} progressively increased during LPD, and, on the last day of this diet (Table 2), both FE_{Na} and C_{H_2O}/C_{In} were doubled with respect to the values detected at the end of NPD. The striking increase in these parameters was associated with no significant change in basal fractional excretion of potassium (FE_K; Table 2). These data, obtained in a state of maximal water diuresis by clearance methods previously described [28–31], strongly suggest that during LPD a decrease in Na reabsorption is proximal to the macula densa. In contrast to CON, in CRF the similarity of GFR and RPF during the two different diets was coupled with unchanged FE_{Na} and C_{H_2O}/C_{In}. In this group, moreover, FE_{Na} values were significantly much higher (above 4%) than in healthy subjects at any dietary protein level while no significant change of FE_K was observed.

In CRF a decreased proteinuria corresponded to the reduction in protein intake. Indeed, the urinary excretion of total

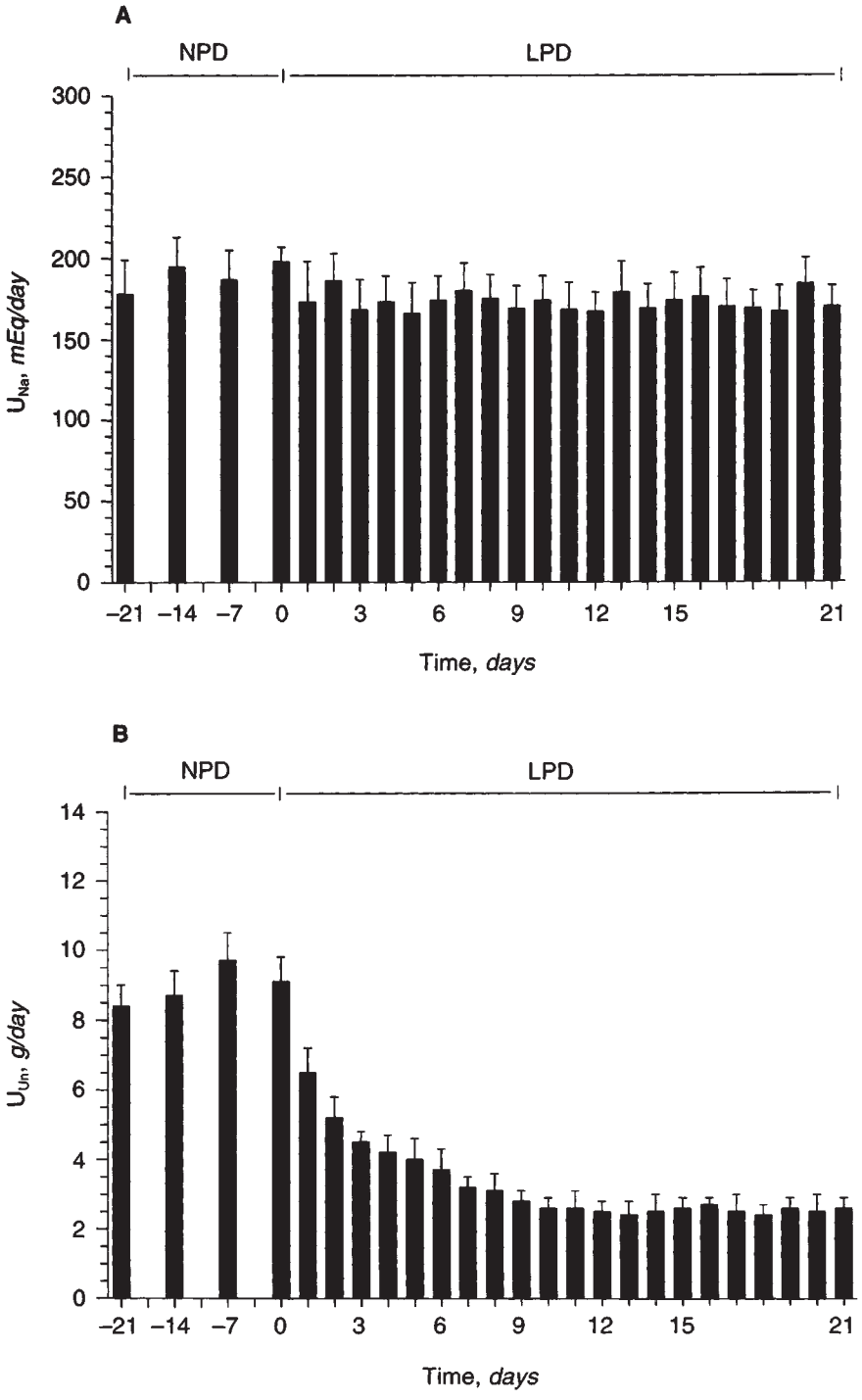

Fig. 1. *Daily urinary sodium (A) and urea nitrogen (B) excretion during normal protein diet (NPD) and low protein diet (LPD) in patients with CRF (N = 10).* The urinary urea nitrogen excretion was significantly lower from the second day of LPD.

protein was 3.31 ± 0.7 g/day at the end of NPD and 1.68 ± 0.4 g/day at the end of the low protein diet ($P < 0.005$).

The changes in blood pressure and renal dynamics three hours after the acute stimulus (OPL + D) are reported in Table 3. In both patients and controls GFR and RPF progressively increased throughout the acute study, reaching the maximal response three hours after the stimulus. At the end of the third hour, RPF and GFR were significantly augmented in both groups at any level of protein intake. At LPD in both CRF and CON, the renal vasodilatory response was characterized by a significant reduction of renal vascular resistance (RVR). In contrast, in the two groups of subjects at NPD, RVR did not change significantly. Such a finding is likely related to the increase in MAP observed after the stimulus in the two groups kept at NPD. The acute stimulus caused an increase of FE_{Na} in all the different conditions (Table 3). Such a phenomenon has

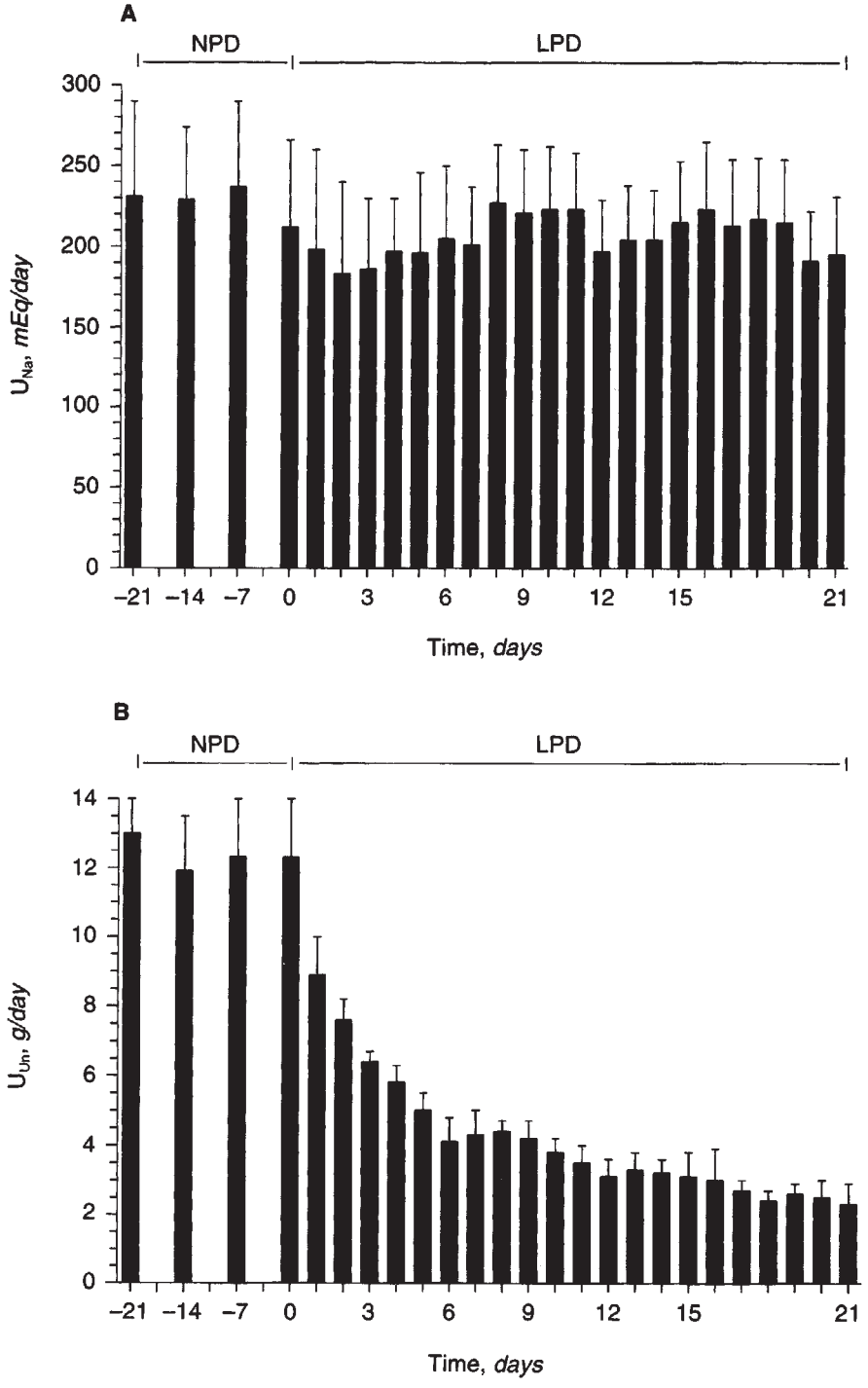

Fig. 2. *Daily urinary sodium (A) and urea nitrogen (B) excretion during normal protein diet (NPD) and low protein diet (LPD) in healthy subjects* (N = 6). The urinary urea nitrogen excretion decreased significantly from the second day of LPD.

been previously described and has been linked to the natriuretic properties of dopamine [19, 32].

The percent increases of GFR and RPF [(stimulated−basal)/basal * 100] are depicted in Figure 4. In controls, ΔGFR was significantly higher at LPD when compared to NPD (31% vs. 24%, respectively, $P < 0.05$), while ΔRPF was 38% at LPD and 35% at NPD (NS). In CRF, the vasodilatory response to the acute stimulus was blunted overall. Indeed, ΔGFR was lower with respect to controls and did not change between the two different conditions, being 17% at LPD and 16% at NPD. Similarly, ΔRPF was 16% at LPD and 12% at NPD. Finally, in healthy subjects only was an inverse correlation found between Δ GFR and basal GFR ($r = -0.6947$, $P < 0.02$).

Discussion

We have previously underlined that patient compliance to protein restriction is difficult to attain mostly in the first weeks of prescription [33], and it is even more demanding to maintain

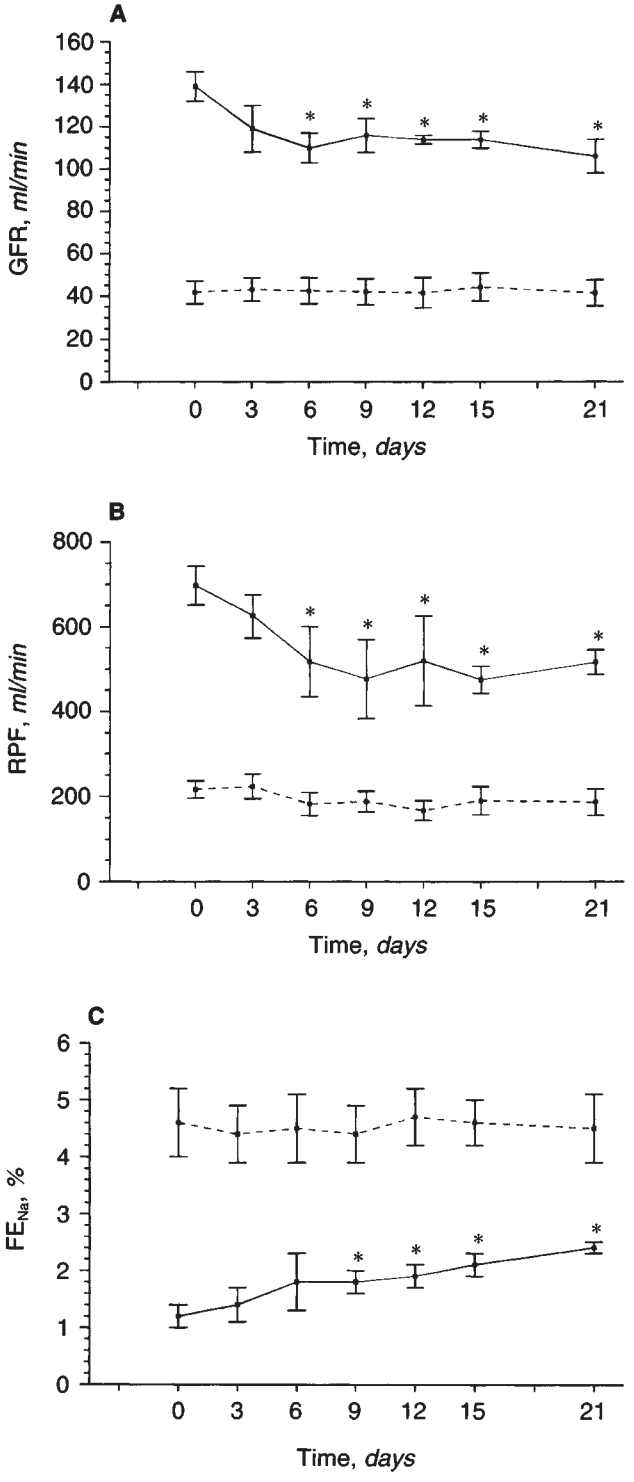

Fig. 3. *GFR (A), RPF (B) and FE_{Na} (C) (—▲—) (N = 6) and CRF (····) (N = 10) on the last day of normal protein diet (day 0) and during the successive three weeks of low protein diet.* * $P < 0.05$ vs. day 0.

sodium intake constant after protein restriction [6, 8, 11]. In the current study, both groups of subjects were compliant to the assigned diet. This was confirmed in the first period (NPD) by the weekly results of the interviews with the dietician and urinary measurements, while in the second period the compliance to LPD was carefully verified on a daily basis by urine collections. Such a compliance was crucial since it permitted an evaluation of the pure renal effects of a reduced protein intake, that is, without changes in sodium intake.

In CRF patients, the chronic study evidenced a constancy of MAP, GFR, RPF and FF despite a reduced dietary protein intake; the unmodified renal dynamics following LPD were associated with a significant decrease in 24-hour urinary excretion of total protein. Interestingly, preliminary data from the recent multicenter Modification of Diet in Renal Disease (MDRD) study have found a decreased GFR in CRF patients in the first four months of treatment with the 0.58 g protein/kg/day diet [34]. In that study a critical question was raised as to whether the initial fall in the glomerular filtration rate is due to a physiological change in renal hemodynamics or to a progression of renal disease. The current data suggest that a reduction in dietary protein intake does not acutely change renal hemodynamics in CRF patients. However, our period of experimental observation was limited to the first three weeks only, and therefore we cannot exclude a subsequent fall in GFR in our patients.

Findings similar to ours have been previously reported by Rosemberg et al in patients with comparable degrees of CRF and systemic blood pressure but kept at a lower level of salt intake (3 to 4 g/day) [1]. In that study the authors demonstrated an improvement in glomerular permselectivity after LPD. They hypothesized that the stability of GFR during protein restriction was likely representative of offsetting changes in glomerular pressure and the glomerular ultrafiltration coefficient with the LPD reducing glomerular pressure and increasing the ultrafiltration coefficient.

As opposed to CRF, in healthy subjects LPD was associated with a decreased renal perfusion witnessed by lower GFR and RPF coupled with higher RVR. Such different renal dynamic responses to dietary protein change may be accounted for by diverse LPD-induced glomerular effects in healthy and disease conditions, the latter being characterized by higher MAP and, likely, by glomerular hyperfiltration and altered glomerular permselectivity [4]. Nevertheless, conclusive data on the glomerular effects of dietary protein cannot be provided since our clinical study does not permit a direct assessment of intrarenal hemodynamics.

On the other hand, the observation of different effects of protein intake on sodium tubular reabsorption at the level of the proximal nephron generates a new alternative hypothesis. Normal subjects presented a marked difference in basal fractional urinary excretion of sodium and free-water between the two diets: in this group, on LPD FE_{Na} increased progressively (Fig. 3c) to such an extent that, at the end of this period, FE_{Na} was doubled when compared with the value recorded on the last day of NPD. Similarly, in the same group the fractional free-water generation (C_{H_2O}/C_{In}) doubled at the end of LPD (Table 2). Therefore, these data obtained during maximal water diuresis strongly suggest an increased distal delivery of sodium during protein restriction in healthy people. No significant change of fractional urinary potassium excretion paralleled the increase in FE_{Na}, further supporting the hypothesis that in CON group LPD was associated to a decreased tubular reabsorption proximal to the macula densa. Conversely, in CRF patients after

Table 3. Renal dynamics in basal condition (Basal) and at the end of third hour from acute stimulus (Stimulated)

		Patients (N = 14)		Controls (N = 6)	
		NPD	LPD	NPD	LPD
MAP *mm Hg*	Basal	97 ± 2[a]	101 ± 4[a]	86 ± 3	86 ± 2
	Stimulated	102 ± 3[ac]	100 ± 3[a]	91 ± 3[c]	87 ± 1
GFR *ml/min*	Basal	38.8 ± 4[a]	39.5 ± 5[a]	139 ± 7	106 ± 8[b]
	Stimulated	45.8 ± 6[ac]	45.6 ± 5[ac]	172 ± 7[c]	138 ± 10[bc]
RPF *ml/min*	Basal	198 ± 16[a]	184 ± 23[a]	697 ± 46	515 ± 29[b]
	Stimulated	219 ± 20[ac]	210 ± 24[ac]	941 ± 56[c]	711 ± 39[bc]
FF %	Basal	20 ± 1	23 ± 2	21 ± 2	21 ± 1
	Stimulated	20 ± 1	23 ± 3	19 ± 2[c]	20 ± 2
RVR *mm Hg/ml/min*	Basal	0.342 ± 0.037[a]	0.413 ± 0.051[a]	0.074 ± 0.002	0.090 ± 0.003[b]
	Stimulated	0.311 ± 0.032[a]	0.349 ± 0.040[ac]	0.070 ± 0.004	0.079 ± 0.002[c]
V *ml/min*	Basal	6.0 ± 0.6[a]	5.3 ± 0.4[a]	9.8 ± 1.4	10.1 ± 2.7
	Stimulated	6.7 ± 0.4[a]	6.8 ± 0.6[ac]	10.9 ± 1.9	13.7 ± 2.0
FE_{Na} %	Basal	4.6 ± 0.6[a]	4.5 ± 0.6[a]	1.2 ± 0.2	2.4 ± 0.1[b]
	Stimulated	5.2 ± 0.8[a]	6.2 ± 0.9[ac]	2.1 ± 0.2[c]	3.1 ± 0.1[bc]

Abbreviations are: GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure; RVR, renal vascular resistance; V, urinary volume; FE_{Na}, fractional urinary sodium excretion.

[a] $P < 0.05$ vs. Control

[b] $P < 0.05$ vs. NPD

[c] $P < 0.05$ vs. basal

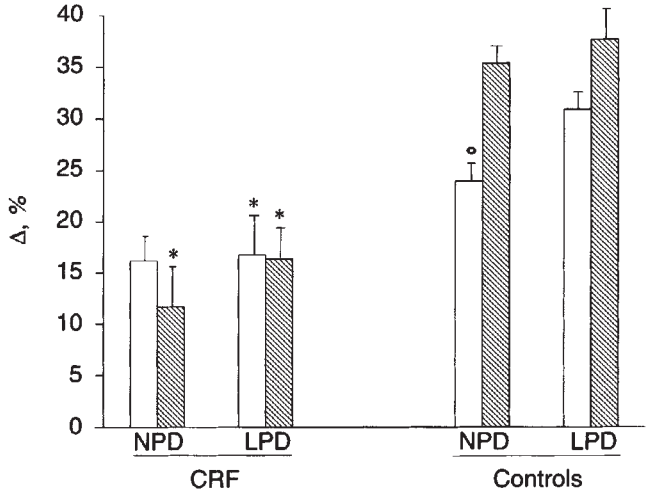

Fig. 4. *Percent increase of GFR (□) and RPF (▨) measured at the end of the third hour after the acute stimulus in control subjects (N = 6) and CRF patients (N = 14) maintained on different protein intakes.* * $P < 0.05$ vs. controls; ° $P < 0.05$ vs. LPD.

LPD, the unchanged renal hemodynamics were associated with an unvaried and marked reduction in proximal sodium reabsorption, since both C_{H_2O}/C_{In} and FE_{Na} remained unmodified, with FE_{Na} values more than doubled in this group when compared to CON, and no significant change of FE_K paralleled the increase in FE_{Na} (Fig. 3c, Table 2).

A possible linkage between glomerular and tubular effects of dietary protein change comes from previous experimental studies. In normal rats, Seney and Wright demonstrated that protein restriction leads to a decreased GFR through activation of the tubuloglomerular feedback system (TGF) secondary to an increased distal delivery of sodium [35]. On the basis of those results, they hypothesized a reduced amino acid-NaCl tubular reabsorption proximal to the macula densa. Similar events may have therefore accounted for the lower values of GFR observed in our CON subjects on LPD, while in CRF the marked reduction of proximal tubular reabsorption may have overridden the normal effects of proteins on tubular reabsorption contributing to the lack of variance in renal dynamics. In CRF patients, the reduced tubular reabsorption in the proximal nephron is likely related to the volume-dependent increase in blood pressure as suggested in previous clinical studies by our group [27, 31].

To gain more insight into the renal effects of protein intake in CRF, we tested renal functional reserve (RFR) by administering an oral protein load (OPL) associated with vasodilating doses of dopamine (D). Such a combined administration was used to achieve the maximal response [19]. The vasodilating stimulus resulted in significant increases of GFR and RPF in both groups at any protein intake level; however, some important differences in the response were detected (Fig. 4). A blunted vasodilatory response was recorded overall in CRF; indeed, in patients, ΔGFR was lower and constant, being 17% at LPD and 16% at NPD, while in controls ΔGFR was significantly higher at LPD than at NPD (31% vs. 24%, respectively).

As hypothesized by Bosch et al, in chronic renal failure RFR declines since glomerular hyperfiltration would compensate for the loss of functioning renal mass [7, 14]. Similarly, studies in healthy subjects by our group have found that the magnitude of the response to amino acid plus dopamine infusion is inversely correlated to the resting GFR [25]. Accordingly, we may interpret the present findings as a consequence of the dependency of RFR from the basal glomerular arteriolar tone. In other words, the lower ΔGFR detected in CON at NPD after the stimulus may be related to the basal glomerular vasodilation induced by the higher chronic protein intake. Likewise, in CRF the presence of glomerular hypertension resulting from reduced renal mass and augmented systemic arterial pressure may be responsible for the blunted response. However, the constancy of RFR response to the diet shifting in patients with CRF raises some concern about the postulated linkage between RFR and glomerular hyperfiltration. Indeed, in this group, the percent increase of GFR and RPF did not increase after protein restriction despite a significant reduction of urinary protein excretion

assumed to be evidence of a decreasing glomerular hypertension [36, 37]. Due to the absence of a direct assessment of glomerular pressure, the reason for such a contradictory result is not readily apparent. However, it is important to note that, according to recent micropucture studies in diverse experimental models of renal disease, the loss of RFR does not necessarily detect the presence of glomerular hypertension while it is constantly associated with abnormalities in proximal tubular reabsorption [15–18]. Interestingly, as for chronic protein loads, an acute amino acid load raises GFR through a TGF mechanism [38], and furthermore, Woods et al have demonstrated that an increased distal delivery of sodium prevents the normal expression of RFR [39, 40]. Specifically, they found loss of RFR following administration of furosemide, whereas, distally acting diuretics did allow a normal response. Moreover, induction of an experimental model of Fanconi syndrome, in which proximal tubular reabsorption was damaged and basal FE_{Na} rose, was associated with an absence of renal reserve. Our data therefore suggest that, in the CRF group the unmodified RFR response to the dietary protein shifting was at least partially related to the significant reduction of basal proximal reabsorption preventing any TGF-dependent change in glomerular perfusion regardless the level of protein intake. In other words, in CRF, besides the presence of glomerular hypertension, a high distal delivery of sodium may affect the response to an acute vasodilating stimulus.

In conclusion, our study provides the first evidence that in patients with moderately severe CRF, the maintenance of a sodium intake above 10 g/day is associated with an abnormal response of basal and stimulated renal function to short-term dietary protein restriction. Although glomerular effects of LPD cannot be excluded, an emerging role in determining the renal response to a low-protein diet appears to be played by tubular reabsorption in the proximal nephron.

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